PROTEIN INTERACTOR DETECTION SYSTEMS

GOVERNMENT SUPPORT

[0001] This invention was made with government support under Grant No. GM105446 and GM054616 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0002] Genome sequences of all important model organisms are now available, owing to the technological advances in high throughput DNA sequencing. An important next step in understanding biology and disease is to identify protein interactome and to characterize protein machines, which carry out every major cellular process and maintain homeostasis of organism (Seebacher et al., *Cell* 144, 1000, 1000.e1. (2011); Bonetta, Nature 468, 851-854 (2010); Perkel, Science 329, 463-465 (2010); and Alberts, Cell 92, 291-294 (1998)). However, unlike the genome, a protein interactome is dynamic and protein machines may include weakly associated components, which hit the blind spots of current technologies.

[0003] Protein-protein interaction networks underlie most cellular processes. Protein machines execute most cell functions, such as the replisome for DNA replication; RNA polymerase complex for DNA transcription; ribosomes for protein synthesis; and anaphase-promoting complex that drives cell cycle progression in both early embryo development and adult homeostasis. To understand biology and disease and to identify new therapeutic targets, it is necessary to search, analyze and visualize protein-protein interaction networks. Indeed, over the past decade especially since completeness of the human genome project, much effort has been devoted to the study of protein interactome. Large databases have been generated, such as BioGRID and IntAct. Now researchers and funding agencies are gearing up to map the human interactome (including protein-protein and protein-DNA interactions). For example, the Canada Foundation for Innovation along with its partners awarded nearly US$20 million "to create a technology platform to map the human interactome."

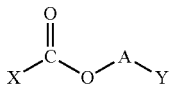
[0004] The previous technologies for mapping protein-protein interactions have blind spots. Affinity purification coupled to mass spectrometry (AP/MS) separates protein complexes from cell extracts, followed by characterization of the components based on mass. Some partners whose interactions depend on cellular environment are thus likely to be missed. While chemical crosslinking may be used, it lacks specificity. The second type of methods detects pairwise protein interactions in a cellular context, including yeast two-hybrid assay (Y2H), protein complementation assay (PCA), luminescence-based mammalian interactome (LUMIER), and mammalian protein-protein interaction trap (MAPPIT). However, each method only detects a small percentage of the interactions (<50%) as demonstrated in a recent study.

[0005] Accordingly, there is a need for new systems, reagents, and methods for the determination of protein-protein interactions.

SUMMARY

[0006] Provided herein are systems, methods and reagents for determining interactors (proteins or nucleic acids) that interact with a protein of interest. The subject system, methods and reagents advantageously allow for the identification of weak and transient protein-protein and protein-interactions. Such subject system, methods and reagents are useful, for example, for the determination of specific protein-interactor interactions that exist in particular diseases. Determination of these differences is useful, for example, in the drug development for the treatment of such diseases.

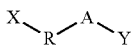
[0007] In a first aspect, provided herein is an interactor detection molecule according to the formula:



[0008] where: X is a label; A is an optional linker; and Y is a reactive group capable of reacting with a cysteine, lysine histidine or serine side chain upon oxidation by a singlet oxygen ($^1O_2$). In some embodiments, the label X is a radioisotope, a stable isotope, a fluorophore, an electron dense metal, biotin, a nucleic acid or an antibody epitope. In certain embodiments, the linker A is an alkyl chain, an aryl, a heteroaryl, or polyethylene glycol.

[0009] In some embodiments, the reactive group Y is a thiol, a furan, a pyrrole, an enol ether, a phenol or a naphthol or derivative. In certain embodiments, the thiol is selected from an alkyl thiol, an aryl thiol, a cysteine and a peptide that includes a cysteine. In certain embodiments, X is biotin, A is CH_2CH_2, and Y is a thiol containing group.

[0010] In another aspect, provided herein is an interactor detection molecule having a formula:



[0011] where X is a label; R is a cleavable peptide; A is an optional linker; and Y is a reactive group capable of reacting with a cysteine, lysine histidine or serine side chain upon oxidation by a singlet oxygen ($^1O_2$). In some embodiments, the label X is a radioisotope, a stable isotope, a fluorophore, an electron dense metal, biotin, a nucleic acid or an antibody epitope. In certain embodiments, the linker A is an alkyl chain, an aryl, a heteroaryl, or polyethyleneglycol. In some embodiments, the cleavable peptide R includes a protease cleavage site. In exemplary embodiments, the protease cleavage site is a Tobacco Etch Virus protease cleavage site. In certain embodiments, the linker A is an alkyl chain, an aryl, a heteroaryl, or polyethylene glycol. In some embodiments, X is biotin and Y is a cysteine.

[0012] In exemplary embodiments, the reactive group is a thiol, a furan, a pyrrole, an enol ether, a phenol or a naphthol or derivative. In some embodiments, the thiol is selected from an alkyl thiol, an aryl thiol, a cysteine and a peptide that includes a cysteine.

[0013] In another aspect, provided herein is a system for determining interactors that interact with a protein of interest comprising: In some embodiments, the SOG-POI protein includes a singlet oxygen photosensitizer linked to a protein of interest, where the singlet oxygen photosensitizer is capable of producing singlet oxygen ($^1O_2$) when illuminated with a light source; and any one of the interactor detection molecules provided herein.